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Original Contribution

Pesticides associated with Wheeze among Commercial Pesticide Applicators in the Agricultural Health Study

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Pesticides are potential risk factors for respiratory disease among farmers, but farmers are also exposed to other respiratory toxicants. To explore the association of pesticides with wheeze in a population without other farming exposures, the authors analyzed data from 2,255 Iowa commercial pesticide applicators enrolled in the Agricultural Health Study. Controlling for age, smoking status, asthma and atopy history, and body mass index, the authors calculated odds ratios for the relationship between wheeze and 36 individual pesticides participants had used during the year before enrollment (1993–1997). Eight of 16 herbicides were associated with wheeze in single-agent models; however, the risk was almost exclusively associated with the herbicide chlorimuron-ethyl (odds ratio (OR) = 1.62, 95% confidence interval (CI): 1.25, 2.10). Inclusion of chlorimuron-ethyl in models for the other herbicides virtually eliminated the associations. The odds ratios for four organophosphate insecticides (terbufos, fonofos, chlorpyrifos, and phorate) were elevated when these chemicals were modeled individually and remained elevated, though attenuated somewhat, when chlorimuron-ethyl was included. The association for dichlorvos, another organophosphate insecticide, was not attenuated by chlorimuron-ethyl (OR = 2.48, 95% CI: 1.08, 5.66). Dose-response trends were observed for chlorimuron-ethyl, chlorpyrifos, and phorate; the strongest odds ratio was for applying chlorpyrifos on more than 40 days per year (OR = 2.40, 95% CI: 1.24, 4.65). These results add to the emerging literature linking organophosphate insecticides and respiratory health and suggest a role for chlorimuron-ethyl.

agriculture; insecticides; occupational exposure; organophosphates; pesticides; signs and symptoms, respiratory; sulfonylurea compounds

Abbreviations: AHS, Agricultural Health Study; CI, confidence interval; OR, odds ratio.

Pesticides may contribute to respiratory symptoms and disease. Surveys of farmers and rural residents have linked pesticides in general and certain pesticide classes in particular with respiratory symptoms, but few studies have identified specific pesticides. In the American Midwest, working with pesticides, applying pesticides to livestock, and applying pesticides aerially have been associated with a higher prevalence of respiratory symptoms (1–3). In Saskatchewan,

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Canada, farmers working with carbamates and organophosphates have reported a higher prevalence of asthma than other farmers (4).

In the Agricultural Health Study (AHS), a longitudinal study of licensed pesticide applicators and their spouses in Iowa and North Carolina, we observed increased odds of wheeze associated with 11 of 40 pesticides used during the past year among more than 20,000 farmer pesticide applicators (5). Pesticides associated with increased wheeze included the herbicides paraquat, atrazine, alachlor, and chlorimuron-ethyl and the organophosphate insecticides parathion and chlorpyrifos. Increased odds of wheeze were associated with increasing days of pesticide application overall, although when we evaluated total days of organophosphate use, we saw no elevation in wheeze risk with increasing days of application. We also observed increased odds of wheeze with more traditional farming exposures, including exposures to animals and tractors (6, 7).

Evaluating the respiratory health effects of pesticides among farmers presents a challenge because of farmers' high exposures to many other respiratory irritants and toxicants, such as animals and grains. To disentangle pesticide exposures from other farm exposures, we used previously unanalyzed data on commercial pesticide applicators from the AHS. As professional pesticide applicators, commercial applicators apply pesticides more frequently than do farmers and have fewer exposures to other agricultural respiratory hazards than do farmers.

MATERIALS AND METHODS

We conducted this cross-sectional analysis of wheeze and pesticide use and other agricultural exposures among commercial pesticide applicators in the AHS. The AHS is a large prospective cohort study of licensed pesticide applicators and their families in Iowa and North Carolina. Participants were enrolled between 1993 and 1997 (8). Participants in the study are primarily farmers and their spouses, but almost 5,000 commercial pesticide applicators in Iowa (47 percent of those licensed in the state) enrolled in the study in the same manner as the farmers. No commercial pesticide applicators were recruited in North Carolina. Commercial applicators who were also certified as private applicators were designated farmers in the AHS and were included in the previous analyses (5–7).

Commercial applicators apply pesticides for hire to land, plants, seed, animals, waters, and structures. They include persons who apply pesticides to lawns, golf courses, and homes for termite control, as well as in agricultural settings. Details on the enrollment of commercial applicators are presented elsewhere (8). Briefly, pesticide applicators enrolled in the study by completing the enrollment questionnaire at the time of pesticide licensing and recertification; 2,375 of the 4,916 commercial applicators (48 percent) returned a second, more detailed questionnaire that included questions on wheeze and asthma history. This analysis is limited to commercial applicators who returned both questionnaires (copies are available at the AHS website (http://www.aghealth.org/questionnaires.html)). The commercial applicators who returned the second questionnaire were more likely to apply and handle pesticides than those who did not return it; we saw no difference between the groups with regard to medical history, smoking, or demographic factors. The AHS has been reviewed and approved by institutional review boards at the National Institutes of Health, the University of Iowa, and Battelle Life Sciences (Durham, North Carolina).

We assessed exposure and outcome using two self-administered questionnaires. The first questionnaire, the enrollment questionnaire, obtained information on 22 pesticides used during the year before enrollment, smoking history, current agricultural activities, and demographic factors. The second questionnaire asked about 18 additional pesticides used during the last year, methods of pesticide application, use of personal protective equipment, use of pesticide additives, and medical history regarding wheeze, asthma, eczema, and hay fever. For this analysis, asthma was defined as a self-report of a doctor's diagnosis of asthma, and atopy was defined as a self-report of a doctor's diagnosis of either eczema or hay fever.

We focused on exposures reported during the year before enrollment because these were temporally relevant for the wheeze outcome. In addition to pesticide information, we used information on animals raised and other farm activities engaged in during the past year. For pesticides, we had information regarding lifetime use of the 40 pesticides that were on the market at the time of enrollment. For these 40 pesticides, participants provided information on ever use, use during the past year, frequency of use, and total number of years used. For each pesticide, exposure was categorized as never, currently, or formerly used. The formerly-used category contained persons who reported using the pesticide but did not indicate that they had used it during the past year. We included both current users and former users in analyses of multiple pesticides in order to assess confounding. We also evaluated other factors related to the application of chemicals, including application methods, use of personal protective gear, and use of solvents as pesticide additives. We evaluated farming activities related to animals and animal production, farm maintenance, and grain handling as potential risk factors for wheeze.

Wheeze in the past year was based on the participant's reply to the question, "How many episodes of wheezing or whistling in your chest have you had in the past 12 months?," with five possible responses—"no wheezing or whistling," "1–2 episodes," "3–6 episodes," "7–12 episodes," and "more than 12 episodes." Any positive response (one or more episodes) was defined as a case of wheeze. We had no other information on respiratory symptoms experienced during the past year.

Pesticides and other agricultural exposures were evaluated using a common base logistic regression model controlling for potential confounders. The base models included five age categories (<31, 31–40, 41–50, 51–60, and ≥ 61 years), smoking history (current, past, never), body mass index (weight (kg)/height (m)^2; <25, ≥ 25), and asthma/atopy status. Since persons with atopy and persons with asthma may differ in their propensity to wheeze, we created a four-category asthma/atopy status variable (both asthma and atopy, asthma alone, atopy alone, neither). We evaluated potential confounding by other smoking variables, such as pack-years of smoking and current smoking habits, using both model-fit and change-in-the-estimate criteria. Parameterization of smoking status as three categories (current, past, and never) provided the best fit, and we observed no evidence of confounding of pesticide estimates with the inclusion of additional smoking variables.

The association of chemical-specific exposures with wheeze in the past year was based on both ever use in the past year and frequency of application. Each of the 40 pesticides was included in the base model individually. We restricted dose-response modeling to insecticides and herbicides, because use of fungicides and fumigants was limited to a few days per year. We used the self-reported category for average number of days applied per year in dose-response models; higher categories were collapsed if there were insufficient numbers of exposed respondents (<1 percent in a cell). We performed chi-squared tests for trend for each chemical using an ordinal term for the questionnaire's days-per-year category. To evaluate the impact of total pesticide usage during the year, we constructed summary variables for total days of use in the past year: 1) total pesticide use (the sum of all herbicides, insecticides, fungicides, and fumigants), 2) total pesticide use within each pesticide class (e.g., herbicides, insecticides, fungicides, and fumigants), and 3) overall annual usage of organophosphate insecticides. The organophosphate class contains chlorpyrifos, coumaphos, diazinon, dichlorvos, fonofos, malathion, parathion, phorate, terbufos, and trichlorfon. The number of days of pesticide application was calculated for each group using the median value for each category or 50 percent above the lower bound of the top category and then summing over all chemicals in that group.

Pesticides are commonly used together; to address this issue, we assessed pairwise correlations between the chemicals that were associated with wheeze in single-agent models. For pairs of currently used pesticides with a Pearson correlation coefficient greater than 0.5, we constructed models including both chemicals. For chemicals with evidence of confounding by other pesticides, we conducted the dose-response analyses again using models containing the dose categories for the chemical of interest and the use status for the potential confounder (current, former, never). We also constructed models with other sets of potentially related exposures, such as animals and pesticides used on animals.

We used the P1REL0310 release of the AHS data set, and all statistical analyses were conducted with SAS software (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Of the 2,375 commercial pesticide applicators who completed both questionnaires, 2,255 (95 percent) had complete information on all base-model covariates (table 1). Subjects were predominantly White males aged 17–83 years at enrollment. Most of the commercial applicators were certified for agricultural weed or insect control, with others being registered for a number of nonagricultural certifications (e.g., turf pest control). Over 21 percent of commercial

TABLE 1. Demographic, medical, and pesticide application characteristics of commercial pesticide applicators in the Agricultural Health Study, by wheeze status, 1993–1997

	Wheeze (*n* = 486)		No wheeze (*n* = 1,769)	
	No.	%	No.	%
Age (years)				
17–30	116	24	348	20
31–40	172	35	599	34
41–50	118	24	500	28
51–60	51	10	221	12
61–83	29	6	101	6
Gender				
Female	26	5	88	5
Male	460	95	1,681	95
Education*				
Less than high school	13	3	42	2
High school	190	40	678	39
More than high school	268	57	1,013	58
Race				
White	479	99	1,745	99
Nonwhite	7	1	24	1
Asthma/atopy status†				
No atopy, no asthma	352	72	1,588	90
Asthma, no atopy	30	6	22	1
Atopy, no asthma	58	12	141	8
Both atopy and asthma	46	9	18	1
Smoking status				
Never smoker	157	32	916	52
Past smoker	130	27	542	31
Current smoker	199	41	311	18
Body mass index‡				
16–<23	61	13	224	13
23–<25	70	14	309	18
25–<27	157	32	574	32
27–<31	105	22	342	19
31–63	93	19	320	18
Currently living or working on a farm	157	32	547	31
Pesticide certification type§				
Agricultural weed control	264	56	931	53
Agricultural insect control	206	43	738	43
Agricultural crop disease control	28	6	106	6
Turf pest control	51	11	202	12
Right-of-way pest control	60	13	284	16
General and household pest control	22	5	85	5
Fumigation	33	7	119	7
Years of applying pesticides¶				
0	22	5	78	5
1	35	8	129	8
2–5	116	25	412	24
6–10	100	22	362	22
11–20	122	26	460	27
21–30	52	11	179	11
>30	15	3	63	4

* Information on education was missing for 51 persons.

† Atopy was defined as a history of doctor-diagnosed eczema or hay fever.

‡ Weight (kg)/height (m)^2.

§ Participants could hold more than one type of certification; only those certifications held by at least 5% of the sample are listed.

¶ Information on years of applying pesticides was missing for 110 persons.

applicators reported having at least one episode of wheezing in the year before enrollment. A history of asthma and/or atopy was more common among persons reporting wheeze (28 percent vs. 10 percent); however, 72 percent of persons with wheeze reported neither asthma nor atopic conditions. The total number of years of pesticide application did not differ between subjects who reported wheeze and subjects who did not. Only one third of the group was currently living or working on a farm.

Exposure prevalences and adjusted odds ratios for current use of 36 of the 40 pesticides evaluated are presented in table 2. We omitted aldicarb, parathion, ziram, and methyl bromide because there were fewer than five exposed cases. Thirteen pesticides (eight herbicides and five organophosphate insecticides) used during the year before enrollment were associated with current wheeze. Among the herbicides, chlorimuron-ethyl had the highest odds ratio (odds ratio (OR) = 1.62, 95 percent confidence interval (CI): 1.25, 2.09). Phorate, an organophosphate insecticide, had the highest odds ratio of all currently used chemicals (OR = 2.87, 95 percent CI: 1.70, 4.84). Wheeze was not associated with use of fungicides, fumigants, or other insecticide classes or use of solvents as pesticide additives (data not shown).

We explored patterns of potential confounding by exposure to multiple chemicals. Many herbicides and a few corn insecticides (terbufos and fonofos) had correlation coefficients greater than 0.5 when evaluated using the ever-use variables. Using two-pesticide models, we identified chlorimuron-ethyl as a confounder of all of the herbicide associations; the odds ratios for the other herbicides were attenuated toward 1.0 when chlorimuron-ethyl was included in the model (table 3). Chlorimuron-ethyl also diminished the odds ratio estimates for all of the organophosphate insecticides except dichlorvos, an insecticide used exclusively on animals. In the ever-use models, controlling for chlorimuron-ethyl did not eliminate the elevated odds ratios for the organophosphates; however, only phorate and dichlorvos continued to have significant odds ratios when chlorimuron-ethyl was included in the model (for phorate, OR = 2.35, 95 percent CI: 1.36, 4.06; for dichlorvos, OR = 2.48, 95 percent CI: 1.08, 5.66). The estimated association between chlorimuron-ethyl and wheeze was not attenuated when other herbicides were included, but it was slightly reduced (OR = 1.45, 95 percent CI: 1.11, 1.91) when the insecticide phorate was included (table 3).

We constructed dose-response models with chlorimuron-ethyl included as a potential confounder; results from the dose-response models for chlorimuron-ethyl and four organophosphate insecticides are presented in table 4. Chlorimuron-ethyl, chlorpyrifos, and phorate showed dose-response trends, with suggestive results for terbufos and fonofos. The results for the organophosphates are consistent with a monotonic increase in wheeze with increasing number of days of application. The frequency of use of dichlorvos was limited, so we were unable to construct dose-response models for this chemical. Summary variables for total days of pesticide use suggested that wheeze increased with increasing pesticide use, but only among those persons applying pesticides more than 60 days per year (data not shown). At the highest level of organophosphate application (60 or more days per year), we saw increased odds of wheeze (OR = 2.72, 95 percent CI: 1.54, 4.81); there was no increased wheeze associated with less frequent use of all organophosphate insecticides combined. Use of protective equipment did not influence the odds of wheeze among commercial pesticide applicators (data not shown).

Some commercial pesticide applicators also engaged in farming-related activities that may contribute to respiratory symptoms (table 5). Raising animals was associated with elevated odds of wheeze; however, only the odds ratio for persons working in egg production was statistically significant (OR = 3.62, 95 percent CI: 1.21, 10.82). Performing veterinary procedures (OR = 1.41, 95 percent CI: 1.06, 1.88), handling stored grain (OR = 1.42, 95 percent CI: 1.14, 1.77), grinding feed (OR = 1.36, 95 percent CI: 1.06, 1.74), and painting (OR = 1.32, 95 percent CI: 1.04, 1.68) were associated with increased wheeze. Persons who reported ever having a job besides pesticide application were more likely to wheeze (OR = 1.54, 95 percent CI: 1.09, 2.17); this variable and the farming-related exposures did not influence the odds ratios observed for the pesticides.

DISCUSSION

Epidemiologic and animal data suggest that organophosphate insecticides play a role in respiratory outcomes (5, 9–12). Among Saskatchewan farmers, higher rates of self-reported asthma were associated with organophosphate and carbamate use in the past 5 years (12). Among Kenyan agricultural workers with acetylcholinesterase inhibition, a marker of recent organophosphate and carbamate exposure, reports of three or more respiratory symptoms were four times more common (11); individual pesticides were not evaluated. In our previous analysis of over 20,000 farmers from the AHS, we observed increased odds of wheeze associated with three organophosphate insecticides (parathion, chlorpyrifos, and malathion); parathion had the highest odds of wheeze among all of the pesticides studied (OR = 1.50, 95 percent CI: 1.04, 2.16) (5). In the current analysis, increased odds of wheeze were found for five organophosphates (chlorpyrifos, dichlorvos, fonofos, phorate, and terbufos); phorate and chlorpyrifos demonstrated dose-response trends. Phorate use was more common among commercial applicators than among farmers, particularly for those using it more than 10 days per year, with 3 percent of commercial applicators using this chemical compared with 1 percent of farmers. Malathion was not associated with wheeze among commercial applicators, and there were too few applicators exposed to parathion to estimate odds ratios.

Organophosphate insecticides are associated with airway hyperreactivity in guinea pigs (9, 10, 13). Until recently, this reaction was attributed to acetylcholinesterase inhibition of the vagal nerve (12, 13). However, recent studies in animal models have suggested a different mechanism. In male guinea pigs, subclinical doses of parathion were found to induce airway hyperreactivity (13). Recently, Fryer and colleagues (9, 10) developed a model of organophosphate-induced airway hyperreactivity in guinea pigs and tested it with three organophosphates (chlorpyrifos, parathion, and diazinon) and permethrin, a pyrethroid insecticide. Their

TABLE 2. Prevalence of pesticide use and pesticide-specific odds ratios for wheeze in the past year among commercial pesticide applicators in the Agricultural Health Study, 1993–1997

Pesticide	Pesticide use and wheeze status												Odds ratio for wheeze and current use‡	95% confidence interval
	Never use*				Former use†				Current use					
	Wheeze		No wheeze		Wheeze		No wheeze		Wheeze		No wheeze			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
Herbicides														
2,4-D§	113	23	417	24	145	30	602	34	225	47	745	42	1.27	0.96, 1.68
Alachlor	246	52	953	56	140	30	443	26	89	19	316	19	1.20	0.88, 1.61
Atrazine	215	44	858	49	130	27	453	26	140	29	449	26	1.38	1.06, 1.80
Butylate	345	72	1,308	75	110	23	379	22	25	5	63	4	1.46	0.86, 2.45
Chlorimuron-ethyl	268	56	1,118	64	72	15	235	13	141	29	398	23	1.62	1.26, 2.10
Cyanazine	249	52	969	57	121	25	367	21	106	22	378	22	1.22	0.93, 1.62
Dicamba	190	40	698	41	123	26	449	26	167	35	569	33	1.11	0.86, 1.43
EPTC§	294	62	1,141	67	113	24	336	20	65	14	231	14	1.20	0.87, 1.67
Glyphosate	85	18	360	20	147	30	531	30	254	52	874	50	1.38	1.03, 1.86
Imazethapyr	260	55	1,064	62	76	16	188	11	138	29	461	27	1.35	1.05, 1.75
Metolachlor	235	50	952	56	105	22	320	19	134	28	441	26	1.37	1.05, 1.78
Metribuzin	272	57	1,095	63	122	25	387	22	87	18	266	15	1.42	1.05, 1.92
Paraquat	348	73	1,370	78	98	20	263	15	34	7	116	7	1.21	0.78, 1.85
Pendimethalin	225	47	957	55	112	23	354	20	144	30	440	25	1.38	1.07, 1.79
Petroleum oil	324	68	1,290	74	75	16	231	13	80	17	218	13	1.47	1.08, 2.01
Trifluralin	229	49	880	52	123	26	420	25	120	25	398	23	1.21	0.92, 1.60
Insecticides														
Organophosphates														
Chlorpyrifos	294	61	1,182	67	106	22	333	19	86	18	245	14	1.47	1.09, 1.99
Coumaphos	453	96	1,627	97	13	3	45	3	5	1	11	1	2.02	0.66, 6.24
Diazinon	353	74	1,263	72	76	16	310	18	49	10	174	10	0.81	0.56, 1.18
Dichlorvos	434	91	1,567	92	33	7	116	7	9	2	21	1	2.48	1.09, 5.64
Fonofos	395	83	1,471	86	53	11	169	10	26	6	62	4	1.78	1.07, 2.98
Malathion	211	44	767	44	186	39	702	40	85	18	282	16	1.06	0.78, 1.45
Phorate	388	81	1,477	85	64	13	219	13	27	6	48	3	2.87	1.70, 4.84
Terbufos	376	80	1,402	82	58	12	197	12	38	8	102	6	1.66	1.09, 2.53
Trichlorfon	445	95	1,594	94	13	3	51	3	11	2	54	3	0.58	0.28, 1.18
Carbamates														
Carbaryl	291	61	1,040	59	130	27	529	30	60	13	182	10	1.12	0.79, 1.58
Carbofuran	399	84	1,411	83	51	11	199	12	25	5	89	5	1.04	0.63, 1.70
Other insecticides														
Lindane	437	91	1,609	93	36	8	110	6	5	1	20	1	0.72	0.24, 2.13
Permethrin (crops)	351	74	1,299	76	62	13	194	11	60	13	207	12	1.13	0.81, 1.57
Permethrin (poultry)	432	91	1,573	92	29	6	87	5	13	3	44	3	1.43	0.73, 2.79
Fumigants														
Aluminum phosphide	404	84	1,519	87	44	9	134	8	34	7	93	5	1.44	0.92, 2.24
Fungicides														
Benomyl	433	90	1,549	89	34	7	134	8	15	3	61	4	0.98	0.53, 1.82
Captan	448	95	1,585	94	17	4	64	4	9	2	46	3	0.81	0.37, 1.77
Chlorothalonil	434	90	1,547	88	20	4	99	6	30	6	111	6	0.96	0.61, 1.51
Maneb	455	95	1,636	94	20	4	77	4	6	1	33	2	0.56	0.22, 1.43
Metalaxyl	444	92	1,559	89	22	5	117	7	15	3	71	4	0.66	0.36, 1.21

* "Never use" was the reference category.
† "Former use" indicates that the participant had used the pesticide previously but not during the past year.
‡ Adjusted for age, smoking status, asthma/atopy status, and body mass index.
§ 2,4-D, 2,4-dichlorophenoxyacetic acid; EPTC, *S*-ethyl dipropylthiocarbamate.

TABLE 3. Evaluation of the potential confounding of pesticide-specific odds ratios for wheeze by chlorimuron-ethyl among commercial pesticide applicators in the Agricultural Health Study, 1993–1997

Pesticide (current use)	Estimate for pesticide alone*		Pesticide estimate with chlorimuron-ethyl included		Chlorimuron-ethyl estimate with pesticide included	
	OR†,‡	95% CI†	OR‡	95% CI	OR‡	95% CI
Herbicides						
Chlorimuron-ethyl	1.62	1.26, 2.10				
Atrazine	1.38	1.06, 1.80	0.91	0.63, 1.31	1.76	1.25, 2.47
Glyphosate	1.38	1.03, 1.86	1.14	0.83, 1.57	1.59	1.21, 2.09
Imazethapyr	1.35	1.05, 1.75	1.03	0.71, 1.50	1.51	1.04, 2.20
Metolachlor	1.37	1.05, 1.78	1.01	0.71, 1.43	1.58	1.12, 2.23
Metribuzin	1.42	1.05, 1.92	0.99	0.66, 1.48	1.60	1.13, 2.27
Pendimethalin	1.38	1.07, 1.79	0.99	0.70, 1.41	1.63	1.15, 2.30
Petroleum oil	1.47	1.08, 2.01	1.18	0.83, 1.66	1.52	1.14, 2.01
Organophosphate insecticides						
Chlorpyrifos	1.47	1.09, 1.99	1.27	0.92, 1.74	1.54	1.18, 2.01
Dichlorvos	2.48	1.09, 5.64	2.48	1.08, 5.66	1.60	1.24, 2.08
Fonofos	1.78	1.07, 2.98	1.46	0.86, 2.46	1.54	1.17, 2.01
Phorate	2.87	1.70, 4.84	2.35	1.36, 4.06	1.45	1.11, 1.91
Terbufos	1.66	1.09, 2.53	1.36	0.87, 2.12	1.50	1.14, 1.96

* Estimates for use of a single pesticide alone were copied from table 2.
† OR, odds ratio; CI, confidence interval.
‡ Adjusted for age, smoking status, asthma/atopy status, and body mass index.

model suggested that organophosphate-induced airway hyperreactivity results from effects of autoinhibitory M2 muscarinic receptors on the parasympathetic nerves in the lung, not from acetylcholinesterase inhibition or dysfunction of M3 muscarinic receptors in airway smooth muscle (9). In some cases, airway hyperreactivity occurred at levels below those which caused acetylcholinesterase inhibition; only parathion showed no observable effect level (10). Airway hyperreactivity was not induced by permethrin, suggesting that the mechanism is limited to organophosphates and does not extend to pyrethroids (10). Whether this model with subcutaneous dosing is relevant for human respiratory exposures remains to be seen. If found to be appropriate, however, it suggests that systemic exposure to organophosphate insecticides results in increased airway responsiveness at levels below those which cause acetylcholinesterase inhibition.

Chlorimuron-ethyl was strongly associated with wheeze among commercial pesticide applicators, and it confounded the associations of all other herbicides and attenuated the estimates for many of the organophosphate insecticides. There is little previous evidence of an association of chlorimuron-ethyl, or herbicides in general, with respiratory outcomes. Among farmers, we previously reported increased odds of wheeze in association with six herbicides, including chlorimuron-ethyl; we also observed a dose-response trend for chlorimuron-ethyl (5). We did not observe confounding of the odds ratio estimates for other pesticides by chlorimuron-ethyl; the odds ratio for chlorimuron-ethyl was 1.14 (95 percent CI: 1.02, 1.24) as compared with the odds ratio of 1.62 observed for the commercial applicators (5). Chlorimuron-ethyl use was almost twice as common among commercial applicators as among farmers, and the frequency of use was greater as well. Chlorimuron-ethyl is a sulfonylurea post-emergent herbicide used for peanuts and soybeans (14). Our results are unlikely to be confounded by peanut or soybean exposure, because peanuts are not produced in Iowa and we observed no effect of soybeans on wheeze among the farmers. Our sample contained too few commercial applicators who raised soybeans to evaluate this relationship. Chlorimuron-ethyl is only available in a dry formulation, which may make it more likely to result in exposure via the respiratory route. We were unable to determine whether chlorimuron-ethyl itself or one of the other ingredients in the pesticide product was responsible for the association with wheeze.

Our previous analysis of 20,000 AHS farmers provided us with an opportunity to evaluate the association of 40 different pesticides with wheeze while controlling for other farming-related exposures, including exposure to animals, grains, and solvents (5). Among the farmers, the odds ratio estimates were low (OR = 1.1–1.5), perhaps because of the high background rate of exposure to other agricultural agents. The 2,255 AHS commercial applicators provided us with a unique opportunity to repeat our analysis in a cohort with similar pesticide information but fewer agricultural exposures; only 31 percent of the commercial applicators reported living or working on farms as compared with 96 percent of the farmers. The commercial applicators also had greater rates of pesticide usage than the farmers, allowing us to more easily assess dose-response effects at higher doses. For example, among the farmers, the highest category for chlorpyrifos use was 20 or more days per year, while among

TABLE 4. Results from selected pesticide-specific dose-response models for wheeze among commercial pesticide applicators in the Agricultural Health Study, 1993–1997

Pesticide and no. of days of use in the past year	Wheeze		No wheeze		Odds ratio*	95% confidence interval	*p*-trend†
	No.	%‡	No.	%‡			
Herbicides							
Chlorimuron-ethyl							0.012
0	340	71	1,353	77	1.00		
<5	15	3	43	3	1.87	0.96, 3.63	
5–9	17	4	58	3	1.38	0.75, 2.52	
10–19	51	11	125	7	1.97	1.34, 2.90	
20–39	41	9	125	7	1.41	0.94, 2.13	
≥40	17	4	46	3	1.50	0.81, 2.79	
Organophosphate insecticides§,¶							
Chlorpyrifos							0.003
0	292	61	1,169	67	1.00		
<5	18	4	66	4	1.00	0.56, 1.80	
5–9	16	3	51	3	1.10	0.58, 2.08	
10–19	13	3	59	3	0.77	0.39, 1.49	
20–39	19	4	38	2	1.96	1.05, 3.66	
≥40	18	4	29	2	2.40	1.24, 4.65	
Fonofos							0.09
0	391	83	1,462	87	1.00		
<5	7	2	16	1	1.46	0.56, 3.86	
5–9	2	0	12	1	0.57	0.11, 2.91	
10–19	6	1	12	1	1.55	0.53, 4.54	
≥20	11	2	21	1	1.94	0.87, 4.35	
Terbufos							0.06
0	372	80	1,391	82	1.00		
<5	3	1	19	1	0.73	0.20, 2.63	
5–9	6	1	15	1	1.36	0.48, 3.86	
10–19	13	3	35	2	1.32	0.65, 2.67	
≥20	16	3	31	2	1.82	0.92, 3.61	
Phorate							0.010
0	387	81	1,473	85	1.00		
<5	5	1	10	1	2.01	0.64, 6.32	
5–9	6	1	9	1	3.67	1.25, 10.80	
≥10	16	3	29	2	2.10	1.04, 4.25	

* All odds ratios were adjusted for age, smoking status, asthma/atopy status, body mass index, and previous use of the pesticide.

† Based on the chi-squared test for trend, using the categories presented.

‡ Percentages do not add up to 100, because the prevalence of previous use is not presented (see table 2).

§ In the organophosphate models, odds ratios were additionally adjusted for past and current use of chlorimuron-ethyl.

¶ Dose-response data for diazinon are not presented because of small numbers.

commercial applicators, it was 40 or more days per year. Although our power to detect small risks was reduced, odds ratio estimates in general were higher among commercial applicators than among farmers. We observed similar patterns with chlorimuron-ethyl and organophosphate insecticides, particularly chlorpyrifos, in both groups. Among farmers, we observed increased odds ratios for several herbicides, and these relationships were not confounded by chlorimuron-ethyl. This lack of confounding among farmers may have been due in part to the larger sample size (>20,000

TABLE 5. Odds ratios for relationships between farming-related exposures and wheeze among commercial pesticide applicators in the Agricultural Health Study, 1993–1997

Exposure	Wheeze in the past year (%)		Odds ratio*	95% confidence interval
	Yes (*n* = 486)	No (*n* = 1,769)		
Animals and animal products				
Beef cattle	13	11	1.34	0.97, 1.86
Dairy cattle	2	2	1.84	0.88, 3.87
Hogs	10	11	1.17	0.82, 1.66
Poultry	2	1	1.58	0.70, 3.56
Sheep	2	2	1.47	0.73, 2.98
Eggs	1	1	3.62	1.21, 10.82
Other animal exposures				
Butchering animals	15	12	1.27	0.93, 1.73
Working in swine areas	14	15	1.11	0.82, 1.52
Working in poultry areas	3	2	1.59	0.78, 3.24
Engaging in veterinary procedures	18	15	1.41	1.06, 1.88
Grain exposures				
Handling stored grain	47	42	1.42	1.14, 1.77
Grinding animal feed	27	24	1.36	1.06, 1.74
Handling stored hay	22	21	1.12	0.86, 1.45
Loading or unloading silage	8	8	1.24	0.83, 1.84
Maintenance activities				
Replacing asbestos brake linings	14	13	1.04	0.76, 1.41
Repairing engines	45	39	1.20	0.97, 1.50
Welding	54	51	1.18	0.95, 1.47
Painting	73	68	1.32	1.04, 1.68

* Odds ratios were adjusted for age, smoking status, asthma/atopy status, and body mass index.

farmers) and the inclusion of North Carolina farmers in the farmer cohort, which increased heterogeneity in pesticide-use patterns.

Most pesticide applicators in the AHS used more than one chemical in a year. Our analysis showed strong evidence of confounding of odds ratio estimates for several pesticides by current use of chlorimuron-ethyl. No other chemical confounded the associations observed for other pesticides. Because of the many potential combinations, we limited our evaluation of confounding to those pesticides associated with wheeze in single-chemical models. We saw no evidence of confounding of pesticide odds ratio estimates by agricultural exposures or by other job history.

Wheeze, a common and characteristic symptom of asthma associated with reversible bronchoconstriction, is one of a constellation of common respiratory symptoms, including cough, phlegm, and shortness of breath. We used one question on wheeze to assign outcome; we did not collect information on other respiratory symptoms experienced during the past year. Self-administered questionnaires are reliable and reproducible regarding respiratory symptoms, particularly wheeze, and a physician's diagnosis of asthma (15, 16). Most other large studies of occupational groups have been conducted similarly (17), but often with more detailed information regarding symptom severity and medication use than the AHS, which was initially designed to study cancer. Wheeze is commonly used in occupational studies to assess potential respiratory hazards (1–3, 11, 17).

To our knowledge, the commercial applicators in the AHS are the largest group of commercial pesticide applicators ever studied with regard to respiratory and other health outcomes. Our sample of 2,255 persons provided detailed information on individual pesticide use and important covariates, such as smoking. The information reported by AHS participants, including the commercial applicators, has demonstrated good reproducibility and reliability for all factors, including pesticide use history (18, 19).

The AHS provides a unique opportunity to explore associations between pesticides and respiratory outcomes. Studies of commercial pesticide applicators are infrequent, yet they provide an opportunity to evaluate relationships for pesticides while limiting coexposure to other agricultural agents. In our analysis of commercial pesticide applicators, we observed increased wheeze associated with specific organophosphate insecticides (chlorpyrifos and phorate) and with the herbicide chlorimuron-ethyl. Chlorimuron-ethyl use explained the elevated odds of wheeze seen with other herbicides among commercial applicators. Investigators from recent animal studies (9, 10, 11, 13) have proposed a mechanism linking organophosphates and airway hyperreactivity at doses below those causing acetylcholinesterase inhibition. Our results add to the emerging literature on organophosphate insecticides and respiratory health and suggest a role for chlorimuron-ethyl.

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REFERENCES

1. Jones SM, Burks AW, Spencer HJ, et al. Occupational asthma symptoms and respiratory function among aerial pesticide applicators. Am J Ind Med 2003;43:407–17.
2. Sprince NL, Lewis MQ, Whitten PS, et al. Respiratory symptoms: associations with pesticides, silos, and animal confinement in the Iowa Farm Family Health and Hazard Surveillance Project. Am J Ind Med 2000;38:455–62.
3. Wilkins JR 3rd, Engelhardt HL, Rublaitus SM, et al. Prevalence of chronic respiratory symptoms among Ohio cash grain farmers. Am J Ind Med 1999;35:150–63.
4. Senthilselvan A, McDuffie HH, Dosman JA. Association of asthma with use of pesticides—results of a cross-sectional survey of farmers. Am Rev Respir Dis 1992;146:884–7.
5. Hoppin JA, Umbach DM, London SJ, et al. Chemical predictors of wheeze among farmer pesticide applicators in the Agricultural Health Study. Am J Respir Crit Care Med 2002; 165:683–9.
6. Hoppin JA, Umbach DM, London SJ, et al. Diesel exhaust, solvents, and other occupational exposures as risk factors for wheeze among farmers. Am J Respir Crit Care Med 2004; 169:1308–13.
7. Hoppin JA, Umbach DM, London SJ, et al. Animal production and wheeze in the Agricultural Health Study: interactions with atopy, asthma, and smoking. Occup Environ Med 2003; 60:3e. (Electronic article).
8. Alavanja MC, Sandler DP, McMaster SB, et al. The Agricultural Health Study. Environ Health Perspect 1996;104:362–9.
9. Fryer AD, Lein PJ, Howard AS, et al. Mechanisms of organophosphate insecticide-induced airway hyperreactivity. Am J Physiol Lung Cell Mol Physiol 2004;286:L963–9.
10. Lein PJ, Fryer AD. Organophosphorus insecticides induce airway hyperreactivity by decreasing neuronal M2 muscarinic receptor function independent of acetylcholinesterase inhibition. Toxicol Sci 2005;83:166–76.
11. Ohayo-Mitoko GJ, Kromhout H, Simwa JM, et al. Self reported symptoms and inhibition of acetylcholinesterase activity among Kenyan agricultural workers. Occup Environ Med 2000;57:195–200.
12. Senthilselvan A, Chen Y, Dosman JA. Predictors of asthma and wheezing in adults. Grain farming, sex, and smoking. Am Rev Respir Dis 1993;148:667–70.
13. Segura P, Chavez J, Montano LM, et al. Identification of mechanisms involved in the acute airway toxicity induced by parathion. Naunyn Schmiedebergs Arch Pharmacol 1999; 360:699–710.
14. Meister RT. Crop protection handbook 2005. Willoughby, OH: Meister Media Worldwide, 2005.
15. Burney PG, Laitinen LA, Perdrizet S, et al. Validity and repeatability of the IUATLD (1984) Bronchial Symptoms Questionnaire: an international comparison. Eur Respir J 1989;2:940–5.
16. Jenkins MA, Clarke JR, Carlin JB, et al. Validation of questionnaire and bronchial hyperresponsiveness against respiratory physician assessment in the diagnosis of asthma. Int J Epidemiol 1996;25:609–16.
17. Kimbell-Dunn MR. Work-related respiratory symptoms in New Zealand farmers. Am J Ind Med 2001;39:292–300.
18. Blair A, Tarone RE, Sandler D, et al. Reliability of reporting on lifestyle and agricultural factors by a sample of participants in the Agricultural Health Study from Iowa. Epidemiology 2002;13:94–9.
19. Hoppin JA, Yucel F, Dosemeci M, et al. Accuracy of self-reported pesticide use duration information from licensed pesticide applicators in the Agricultural Health Study. J Expo Anal Environ Epidemiol 2002;12:313–18.